

DOCKET NO.: 206445US0CONT

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

IN RE APPLICATION OF: :

Etsuko MATSUNAGA, et al. :

SERIAL NO.: NEW APPLICATION :

FILED: HERewith :

FOR: METHOD FOR INTRODUCING A GENE INTO A PLANT USING AN  
ADVENTITIOUS BUD REDIFFERENTIATION GENE UNDER THE CONTROL OF  
A LIGHT-INDUCIBLE PROMOTER AS A SELECTABLE MARKER GENE, AND  
VECTOR FOR INTRODUCING A GENE INTO A PLANT USING THE SAME

**PRELIMINARY AMENDMENT**

ASSISTANT COMMISSIONER FOR PATENTS  
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, please amend the above-identified application as follows. Pursuant to 37 C.F.R. §1.121(c)(3), Applicants provide a clean copy of all pending claims below and append a “Marked-up” copy of the amended claims at the end of this amendment.

**IN THE CLAIMS**

--1. (Amended) A method for introducing a gene into a plant, which comprises:

(A) introducing a gene into a plant cell using a vector comprising an adventitious shoot redifferentiation gene as a selectable marker gene under the control of a light-inducible promoter, and

(B) culturing said plant cell into a tissue and selecting a transgenic tissue expressing said adventitious shoot redifferentiation gene, and

(C) regenerating a plant from said transgenic tissue.

2. (Amended) The method according to Claim 1, wherein said transgenic tissue is

selected using, as an index, morphology of an adventitious shoot redifferentiated by expression of the adventitious shoot redifferentiation gene which has been introduced into the plant cell.

3. (Amended) The method according to Claim 1, wherein the light-inducible promoter is a promoter of a ribulose 2-phosphate carboxylase small subunit gene.

4. (Amended) The method according to Claim 1, wherein the adventitious shoot redifferentiation gene is a cytokinin-related gene.

5. (Amended) The method according to Claim 4, wherein the cytokinin-related gene is a CK11 gene.

6. (Amended) A vector for introducing a gene into a plant, comprising a desired gene, an adventitious shoot redifferentiation gene as a selectable marker gene under the control of a light-inducible promoter, and a removable DNA element, wherein the selectable marker gene is positioned such that it behaves integrally with the removable DNA element, and wherein the desired gene is positioned such that it does not behave integrally with the removable DNA element.

7. (Amended) The vector according to Claim 6, wherein the selectable marker gene is present within the removable DNA element.

8. (Amended) The vector according to Claim 6, wherein the light-inducible promoter is a promoter of a ribulose 2-phosphate carboxylase small subunit gene.

9. (Amended) The vector according to Claim 6, wherein the adventitious shoot redifferentiation gene is a cytokinin-related gene.

10. (Amended) The vector according to Claim 9, wherein the cytokinin-related gene is a CK11 gene.

11. (Amended) The vector according to Claim 6, wherein the removable DNA element is derived from a site-specific recombination system.--

Please add new Claims 12 - 26.

- 12. (New) A plant cell to which the vector of Claim 6 has been introduced.
13. (New) A transgenic plant regenerated from the plant cell of Claim 12.
14. (New) A plant cell into which the vector of Claim 6 has been introduced, wherein said vector has lost the removable DNA element and the selectable marker gene.
15. (New) A transgenic plant regenerated from the plant cell of Claim 14.
16. (New) A method for introducing a desired gene into a plant comprising:
- (A) introducing the vector of Claim 6 into a plant cell,
  - (B) culturing said plant cell into a tissue under conditions suitable for detecting morphologically abnormal plant tissue,
  - (C) selecting at least one cell of said morphologically abnormal plant tissue comprising the desired gene and
  - (D) regenerating a plant from said cell.
17. (New) A transgenic plant produced by the method of Claim 16.
18. (New) A method for introducing a desired gene into a plant comprising:
- (A) introducing the vector of Claim 6 into a plant cell,
  - (B) culturing said plant cell into a tissue under conditions suitable for detecting morphologically abnormal plant tissue,
  - (C) selecting at least one cell of said morphologically abnormal plant tissue comprising the desired gene,
  - (D) culturing at least one cell of said morphologically abnormal plant tissue into a tissue under conditions suitable for detection of normal plant tissue,
  - (E) selecting at least one cell of said morphologically normal plant tissue comprising the desired gene and
  - (F) regenerating a plant from said cell.
19. (New) A transgenic plant produced by the method of Claim 18.

20. (New) A method for producing a transgenic plant free from the influence of a selectable marker gene, comprising:

- (A) introducing the vector of Claim 6 into a plant cell,
- (B) culturing said plant cell into a tissue under conditions suitable for detecting morphologically abnormal plant tissue,
- (C) selecting at least one cell of a said morphologically abnormal plant tissue and culturing it into a tissue under conditions suitable for detecting morphologically normal plant tissue,
- (D) selecting at least one cell of said morphologically normal plant tissue, and
- (E) growing at least one cell of said morphologically normal plant tissue into a transgenic plant.

21. (New) A transgenic plant produced by the method of Claim 19.

22. (New) A method for improving redifferentiation efficiency of a transgenic tissue, which comprises introducing a gene into a plant cell using a vector comprising an adventitious shoot redifferentiation gene as a selectable marker gene under the control of a light-inducible promoter.

23. (New) The method according to Claim 21, wherein said transgenic tissue is selected using, as an index, morphology of an adventitious shoot redifferentiated by expression of the adventitious shoot redifferentiation gene which has been introduced into the plant cell.

24. (New) The method according to Claim 21, wherein the light-inducible promoter is a promoter of a ribulose 2-phosphate carboxylase small subunit gene.

25. (New) The method according to Claim 21, wherein the adventitious shoot redifferentiation gene is a cytokinin-related gene.

26. (New) The method according to Claim 25, wherein the cytokinin-related gene is a CKI1 gene.--

#### **REMARKS**

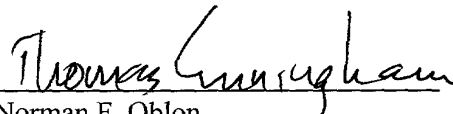
Claims 1-26 are pending. Minor editorial changes have been made to Claims 1-11, such as capitalizing the word "Claim". Claim 1 has been amended to recite a culturing and selection step and a plant regeneration step. Support for this amendment is found throughout the specification and the original claims, e.g. on pages 23-26 of the disclosure and original Claims 1-2. Support for the term *CKII* appearing in Claims 5, 10 and 26 is found *inter alia* in the paragraph bridging pages 11-12 of the disclosure. Accordingly, the Applicants do not believe that any new matter has been introduced.

### CONCLUSION

In view of the above amendments and remarks, the Applicants submit that the claims are now ready for early examination on the merits.

Respectfully submitted,

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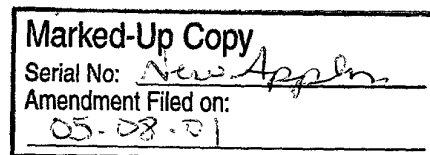
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**MARKED-UP COPY OF CLAIMS**

--1. (Amended) A method for introducing a gene into a plant, which comprises:

(A) introducing a gene into a plant cell using a vector [containing] comprising an adventitious shoot redifferentiation gene as a selectable marker gene under the control of a light-inducible promoter, and

(B) culturing said plant cell into a tissue and selecting a transgenic tissue expressing said adventitious shoot redifferentiation gene, and

(C) regenerating a plant from said transgenic tissue.

2. (Amended) The method according to [c]Claim 1, [which further comprising selecting a] wherein said transgenic tissue is selected using, as an index, morphology of an adventitious shoot redifferentiated by expression of the adventitious shoot redifferentiation gene [which is the selectable marker gene] which has been introduced into the plant cell.

3. (Amended) The method according to [c]Claim 1, wherein the light-inducible promoter is a promoter of a ribulose 2-phosphate carboxylase small subunit gene.

4. (Amended) The method according to [c]Claim 1, wherein the adventitious shoot redifferentiation gene is a cytokinin-related gene.

5. (Amended) The method according to [c] Claim 4, wherein the cytokinin-related gene is a CKII gene [an *ipt*, isopentenyl transferase, gene which is present in a microorganism belonging to the genus *Agrobacterium*].

6. (Amended) A vector for introducing a gene into a plant, [which comprises] comprising a desired gene, an adventitious shoot redifferentiation gene as a selectable marker gene under the control of a light-inducible promoter, and a removable DNA element, wherein the selectable marker gene is positioned such that it behaves integrally with the removable DNA element, and wherein the desired gene is positioned such that it does not behave integrally with

the removable DNA element.

7. (Amended) The vector according to [c]Claim 6, wherein the selectable marker gene is present within the removable DNA element.

8. (Amended) The vector according to [c]Claim 6, wherein the light-inducible promoter is a promoter of a ribulose 2-phosphate carboxylase small subunit gene.

9. (Amended) The vector according to [c]Claim 6, wherein the adventitious shoot redifferentiation gene is a cytokinin-related gene.

10. (Amended) The vector according to [c] Claim 9, wherein the cytokinin-related gene is a CKII gene [an *ipt*, isopentenyl transferase, gene which is present in a microorganism belonging to the genus *Agrobacterium*].

11. (Amended) The vector according to [c]Claim 6, wherein the removable DNA element is derived from a site-specific recombination system.--

Please add new Claims 12 - 26.

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